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Whole mount immunofluorescence microscopy of mouse E7.5 embryo for cilia stain

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An abbreviated version of this protocol was published in Science Advances in Jul 2020

Role of Ca2+ transients at the node of the mouse embryo in breaking of left-right symmetry

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Detailed protocol

1. Recover mouse embryos (E7.5) into 1 ml PBS
2. Remove PBS and fix them in 1 ml 4% PFA solution at 4°C for 30 min.
   *temperature should be modified depending on antigen. For some antigen, room temperature or warmer temp might be better.
3. Wash twice with 1 ml PBT.
4. Permealize the embryos with 1 ml ice-cold methanol (10 min, -20°C) or with 1 ml 0.2% Trion X-100/PBS (10 min on ice)
   *Fixation and permeabilization condition should be modified depending on antibody/antigen.
5. Wash twice with 1 ml PBT.
   *Fixed embryos could be very sticky. The use of PBT is recommended.
6. Block with 500 ul blocking solution at 4°C for 1hr.
7. Add 100 ul 1st antibody in blocking solution with optimized concentration.
   *volume should be modified depending on the number of embryos.
8. Keep at 4°C overnight with rocking gently (or 1hr at room temperature).
9. Wash 6 times with 1 ml PBT. 1hr for each (or can perform o/n washing) with rocking gently at 4°C.
10. During the wash, change the tube to reduce contamination. Wash 1 time with 500 ul blocking solution.
11. Add 100 ul 2nd antibody in blocking solution with optimized concentration.
12. Keep at 4°C overnight with rocking gently (or 1hr at room temperature).
13. Wash 1 time with 1 ml PBT for 1hr at 4°C.
14. During the wash, change the tube to reduce contamination. Wash 1 time with 500 ul blocking solution.
15. Wash 5 times with 1 ml PBT. 1hr for each (or can perform o/n washing) with rocking gently at 4°C.
16. Add PBT and keep it until use at 4°C.
17. Observe the embryonic node with fluorescence microscopy by mounting the excised node onto a glass slide.
    *For excision, we use a hand-made knife made of a needle sharpened by a grinder. For mounting, we use a silicone rubber spacer. See Okada and Hirokawa, Methods Cell Biol., 2009 for detail.

PFA solution

4% Paraformaldehyde in PBS

PBT

0.1% Triton X-100 in PBS

Blocking solution *Can be changed depending on the antibody. We generally use below.

TNB Buffer (0.1M Tris-HCl (pH=7.5), 0.15M NaCl, 0.5% TSA Blocking reagent (PerkinElmer, FP1020))

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Mizuno, K. (2020). Whole mount immunofluorescence microscopy of mouse E7.5 embryo for cilia stain. Bio-protocol Preprint. bio-protocol.org/prep543.
2. Mizuno, K., Shiozawa, K., Katoh, T. A., Minegishi, K., Ide, T., Ikawa, Y., Nishimura, H., Takaoka, K., Itabashi, T., Iwane, A. H., Nakai, J., Shiratori, H. and Hamada, H.(2020). Role of Ca2+ transients at the node of the mouse embryo in breaking of left-right symmetry . Science Advances 6(30). DOI: 10.1126/sciadv.aba1195